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The Detection and Determination of Antimony by Means of Rhodamine B.

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THE DETECTION AND DETERMINATION OF ANTIMONY BY MEANS OF RHODAMINE B

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ABSTRACT

A method for the detection and determination of antimony has been developed, based on the extractability of antimony (III) as the iodide into benzene. The benzene extract is subsequently reacted with an aqueous solution of Rhodamine B, the violet complex of antimony-Rhodamine B forming in the benzene layer.

As a spot test for antimony, the procedure is specific and sensitive. Potential interferences are removed by conditioning the test solution with sodium sulfite and urea. The test conditions are not critical. A complete study of interferences has been made. The limit of identification is 0.2 microgram of antimony at a concentration limit of 1:300,000.

The spectrophotometric procedure, based on the same reactions, permits the determination of microgram quantities of antimony. The method is practically specific, bismuth interfering only if present in high concentrations. An interference study of the same scope as above has been made. The relative analysis error for ten micrograms of antimony is ± 5.0%. The conditions of acidity and reagent concentrations have been studied and have been shown to have wide ranges of tolerance. The color formed is stable for at least three and one-half hours.

A polarographic study of the mechanism indicates that antimony is extracted in the trivalent state.

INTRODUCTION

In recent years the detection and determination of small amounts of antimony has come to be a matter of considerable practical importance.

Antimony, which has been known since at least 3000 B.C., is widely distributed, both in nature and in man-made products. Many thousands of pounds of it go each year into the production of hard lead (12% antimony), used in the production of storage batteries, bullets and shrapnel. It is an important constituent of bearing metal and of type metal, and widely used as a pigment in the paint and rubber industries. Its compounds are often used in the treatment of tropical diseases, thus frequently necessitating its determination in biological samples.

Despite these facts, the trace chemistry of antimony has never been well developed. The writer, upon observing that microgram quantities of antimony (III) as the iodide could be extracted into benzene, has carried out the present study in an attempt to ameliorate this situation.

REVIEW OF THE LITERATURE

A variety of reagents have been proposed for the detection of antimony. Ekkert (12) reported that a large number of phenols, including the cresols, orcinol, phenol, phloroglucinol, and thymol, give color reactions with chloroform solutions of antimony pentachloride. Gutzeit (27) noted that anthracene reacted with a solution of antimony trichloride in carbon tetrachloride to give a green coloration, while indene reacted with the pentachloride in carbon tetrachloride, yielding a red precipitate.

Feigl (19, 20, 21), using pyrogallol as a reagent for antimony, was able to detect as little as four milligrams of the trivalent ion in a liter of solution. He also amassed evidence for the structure of the compound formed. A different structure and a modification of Feigl's procedure were proposed by Takagi and Nagasi (45, 46), after their study of the pryogallol test for antimony. Gallic acid was found by Rossi and Serantes (40) to be useful in the detection of antimony in the presence of bismuth. More recently Wenger, Duckert and Blancpain (10, 51, 52) have recommended fluorone (9-methyl-2, 3, 7-trihydroxy-6-fluorone) as a sensitive reagent for antimony (III). At pH 4.0 they were able to detect as little as 0.2 microgram of antimony. They have also elucidated the structure of the compound formed, and studied the interferences which occur. Unfortunately the reagent is not stable and is exceedingly difficult to prepare; careful control of pH is a critical part of the procedure. Oxine was observed by Pirtea (39) to give a yellow precipitate with as little as twelve micrograms of antimony trichloride in five

milliliters of solution, but the procedure suffers from many interferences.

Ammonium dithiocarbamate is a sensitive reagent for antimony, according to Heller and Macheck (29), but there are many interfering ions. The same was found to be true of cyclohexylethylaminedithiocarbamate by Herrmann-Gurfinkel (30), and by Dubsky and Nejevova (9). Naiman (38) used 2-methylbenzothiazole as a drop reagent for antimony, while Dubsky, Okac and Trtilek (8), found that 2, 5-dimercapto-1, 3, 4-thiodiazole could detect as little as one microgram of antimony; again there were many interferences. Thiourea was observed by Yoe and Overholser (55) to give a pale yellow precipitate, suitable for the detection of antimony; some twenty ions interfered with their procedure.

Caille and Viel (1) discovered the reaction, which now bears their name, in which antimony (III) reacts with a basic nitrogenous compound and potassium iodide to give a yellow color or precipitate, suitable for the microscopical detection of antimony. They investigated a large number of nitrogenous compounds as reagents, including quinoline, antipyrine, pyridine, cinchonine, espartine, morphine, quinine, strychnine, veratrine, and cocaine. There are many interferences to their procedure, bismuth being among the most serious. Martini (36) advocated the use of caffeine as the nitrogenous compound for this procedure.

A number of lake-forming dyestuffs have been investigated as potential reagents for antimony. Smith and Rogers (43) proposed Bismark Brown, Bordeaux Red, Hoffman Violet 3R and phenosafranine, while Dubsky (7) used gallocyanine as a reagent. None of these dyes proved to be satisfactorily sensitive, although gallocyanine was highly selective in a strongly acidic medium. The familiar dyestuff morin was observed by Goto (26)

to give an exceedingly delicate fluorescence test with antimony (III), capable of detecting as little as 0.05 microgram. Unfortunately, as is true with so many procedures involving fluorescence, there are many ions which interfere.

Rhodamine B, a xanthone dyestuff, was in 1927 discovered by Eegriwe (11) to react with solutions of pentavalent antimony, the red color of the reagent being thereby changed to violet or blue, sometimes with the formation of a finely divided precipitate. The reagent as used by Eegriwe was not specific, or even selective, as it could also be used for the detection of bismuth, cobalt, columbium, gold, manganese, mercury, molybdenum, tantalum, thallium, and tungsten. It was, however, a highly sensitive reagent, easily prepared, and stable in solution. As such, it was rather widely used as a confirmatory test for antimony in systematic schemes of analysis (4, 5, 47). With this reagent Eegriwe was able to detect 0.5 microgram of antimony (V) in the presence of even 12,500 times as much tin. In the procedure employing aqueous media the presence of chloride ion is absolutely necessary; ordinarily the test solution is made strongly acidic with hydrochloric acid. The antimony, if present in the trivalent state, must be oxidized, ordinarily with sodium nitrate, before making the test. The composition of the reaction product is not known. (50).

Feigl (16) appears to regard only five tests for antimony as worthy of mention. In addition to the Rhodamine B and fluorone procedures, he describes the test by reduction to the metal, the luminescence test, and the test employing phosphomolybdic acid as a reagent. The first of these (17) has poor sensitivity; the latter two (6, 22) are of excellent

sensitivity, but suffer from a lack of selectivity.

Since the appearance of Feigl's (16) book, one good spot test procedure for antimony has appeared, namely the one of West and Conrad (54), which employs a solution of gossypol in acetone as a reagent. This procedure is very sensitive and highly selective, but does have certain disadvantages; the acidity of the test solution must be carefully controlled and the reagent is no longer readily available.

Very recently Heinrich (28) has introduced an important modification of the Rhodamine B procedure, in which he extracts antimony (V) from 2 N hydrochloric acid solution into diethyl ether, then reacts the ether layer with an aqueous solution of Rhodamine B. Tin, bismuth, gold, thallium, iodide and thiocyanate interfere with the procedure.

Unfortunately the available methods for the determination of microgram quantities of antimony are in no better state than the means for detecting them. No less an authority than Sandell (41) has stated bluntly that "the trace chemistry of this element (antimony) is not well-defined."

Up to the past decade the well-known iodide reaction had for many years formed the basis for most colorimetric determinations of trace amounts of antimony. In this method (15, 31, 37) antimony (III) in strongly acidic solution is treated with excess iodide ion to form the highly colored (Yellow) potassium tetraiodoantimonate (III) complex. Ascorbic acid or hypophosphite is present to reduce small amounts of free iodine which may be liberated. Many oxidizing anions can interfere. Bismuth gives a similar color, but its contribution to the optical density can be subtracted if a reagent of a certain potassium iodide

concentration is used. The reaction is carried out in 10% sulfuric acid solution.

Clarke (2) and others (14, 42) have used the golden yellow color formed with pyridine and an iodide in acid solution for the determination of antimony. The optimum color development occurs in 1:3 sulfuric acid. The concentration of iodide and of pyridine are critical. Fairly small amounts of arsenic and tin do not interfere, but bismuth, zinc and several other metals give colored precipitates. Any pentavalent antimony present is reduced to the trivalent form, which reacts. Sulfurous acid is added to the reagent to reduce any free iodine which might be liberated. Gum arabic is used to keep the complex formed in suspension.

Vasilev and Shub (48) have modified the pyridine-iodide procedure by extracting the developed color into amyl alcohol. This step increases the sensitivity of the method.

An old method (13) for the estimation of antimony utilized a colloidal suspension of the orange-colored sulfide. Any pentavalent antimony was reduced beforehand with sulfur dioxide. This method gave fair accuracy, but only if all other metallic constituents had been removed prior to the determination. Gum arabic was used to stabilize the suspended sulfide.

The familiar molybdenum blue reaction has been applied to the determination of antimony (32), the reaction being that of antimonous ion on phosphomolybdic acid. Iron, copper and tin interfere if present in small amounts. The pH of the system requires careful control.

The reaction of trivalent antimony with phosphomolybdotungstic acid has also been used for its colorimetric estimation (34).

In the absence of arsenic, antimony may be evolved as stibine and estimated by the intensity of the stain formed on mercuric chloride paper (3). This method has been widely used.

Frederick (24) was apparently the first to utilize Rhodamine B as a reagent for the determination of antimony. His procedure involved cooling the reacted system to below 10° C., at which point the antimony-Rhodamine B complex was insoluble, and destroying the excess reagent with bromine. This was a critical operation. The complex was then dissolved in alcohol for the colorimetric estimation. A carefully determined blank was essential, and the method required an experienced operator, as "about 4% of all analyses" failed in Frederick's hands.

Webster and Fairhall (49) provided a considerable advance over Frederick's work by introducing the use of benzene to extract the metal-dye complex after its formation in the aqueous solution, thus shortening the procedure and making it considerably more reliable.

Maren (35) utilized the work of Webster and Fairhall (49) in developing his procedure for the determination of antimony in biological tissues. In addition, he proposed an important alternative method wherein antimony, in the pentavalent state, is extracted into isopropyl ether. The ether layer is removed and shaken with an aqueous solution of Rhodamine B, whereupon the colored complex is developed in the ether, and measured photometrically. In isopropyl ether the complex does not obey Beer's Law, and the color is not stable for more than thirty minutes, but the extraction procedure aids materially in gaining specificity. Maren studied only eleven metals as potential interferences. At 1 milligram arsenic, tin and iron interfered. The interference of iron was eliminated by using the ether method.

The procedure of Maren has been studied by Freeman (23), who noted that the concentration of sulfuric acid in the solution to be extracted exerted a marked effect on the color intensity of the resultant extract. Gelhorn and others (25) have applied the method of Maren to the estimation of antimony in clinical work.

EXPERIMENTAL

I. Materials and Apparatus

0.1000 gram of metallic antimony (CP grade, Baker and Adamson) was carefully weighed, dissolved in 25 milliliters of hot concentrated sulfuric acid, allowed to cool, and diluted to 100 milliliters with water. It was then made up to one liter with 1:3 sulfuric acid. More dilute standards were made up from this solution by pipetting the appropriate volumes into 100 milliliter volumetric flasks and diluting to volume with 1:3 sulfuric acid. These are the standards recommended by Snell and Snell (44) for the colorimetric determination of antimony.

10% potassium iodide solution was prepared by dissolving 10 grams of the CP salt and diluting to 100 milliliters with water.

CP benzene was used without further purification.

1:3 sulfuric acid was prepared by dilution of the concentrated CP shelf reagent with the appropriate volume of water.

The Rhodamine B solution was prepared by dissolving 0.200 grams of the dyestuff in water and diluting to 100 milliliters with water. This solution was filtered before using.

Calibrated weights and volumetric glassware were used throughout the work.

A Beckman Model B spectrophotometer was used for the greater part of the work described herein.

A Beckman Model DU spectrophotometer was used to check critical points in the work. The same Corex cells, of 1.00 centimeter path length, were used in both instruments.

A Sargent Model XII polarograph was used in the polarographic study of the mechanism of the extraction. A Bausch and Lomb large Littrow spectrograph was used in the spectrographic survey of the extraction.

II. The Spot Test Detection of Antimony

In the course of a search for a suitable solvent for the extraction of the potassium tetraiodoantimonate (III) complex, the author observed that the strong yellow color of the complex disappeared when aqueous (acidic) solutions of it were shaken with benzene. Upon removing the benzene layer and shaking it with an aqueous solution of Rhodamine B, the violet-colored complex of antimony-Rhodamine B was observed to form in the benzene. This was obviously a reaction well worth further study, from both a qualitative and a quantitative standpoint.

The initial test solution was one of antimony trichloride in 1 N hydrochloric acid, prepared so as to give a concentration of five micrograms of antimony (III) per drop. One drop of this solution was diluted in a test tube with five drops of 1 N hydrochloric acid, a drop of 10% potassium iodide solution was added, and the aqueous solution was then extracted by vigorously shaking with one milliliter of benzene. Upon shaking the yellow color of the $KSbI_4$ complex was observed to disappear. The benzene layer was then removed with a pipet and put into a second test tube. To this tube was added a drop of a 0.2% aqueous solution of Rhodamine B, and the tube was then vigorously shaken. The characteristic violet hue of the antimony-Rhodamine B complex was seen to appear at once in the benzene layer. A blank of one drop of 1N HCl, treated similarly, produced no color in the benzene layer.

A study of the limit of identification of this procedure indicated that as little as 0.2 microgram of antimony could be detected. An interference study was then begun; the first compound tested, sodium nitrate, imparted to the benzene layer a strongly fluorescent bluish color, which completely masked the test color. As sodium chloride displayed no such behavior, it was obvious that nitrate was the interfering ion. This was most serious, in view of the widespread occurrence of nitrate.

An obvious method of attack was to remove the nitrate by adding sulfuric acid and boiling down to fumes of sulfur trioxide, but the volatility of antimony trichloride precluded this step. The addition of a precipitant for nitrate, such as Nitron, would have made the procedure too cumbersome.

Every prior investigator of the antimony-Rhodamine B reaction had reported the presence of a large concentration of chloride ion as absolutely essential to the formation of the complex. The writer, in the hope that iodide might serve as an acceptable substitute for chloride, prepared standards of (metallic) antimony in 1:3 sulfuric acid. To one drop of such a standard, containing ten micrograms of antimony (III), were added five drops of 1:3 sulfuric acid and one drop of 10% potassium iodide. The solution was then extracted with one milliliter of benzene, with vigorous shaking. The benzene layer was removed and to it was added one drop of the 0.2% aqueous Rhodamine B solution. Upon shaking, the color of the antimony-Rhodamine B complex appeared in the benzene, despite the fact that chloride ion had been nowhere present in the procedure.

It was then a simple matter to remove the interfering nitrate by heating the test solution to fumes of sulfur trioxide, adding water to regain approximately the initial volume, and proceeding as above with the addition of the potassium iodide and subsequent steps.

Later it was found that the interference ascribed to nitrate could be more expeditiously removed by the addition of a few milligrams of solid urea to the test solution. Inasmuch as urea is known to reduce nitrites but not nitrates, this makes it appear likely that the interference was due to the small amount of nitrite present as an impurity in nitrates. The addition of urea was also completely effective in removing the interference due to nitrite as such.

As anticipated, strong oxidizing agents such as permanganate and bromate interfered with the procedure, as described above, by the liberation of free iodine. Upon extraction the benzene layer was so highly colored by the iodine that the test color was completely masked. This difficulty was circumvented by allowing the oxidation of iodide to proceed to completion, and then subsequently reducing the liberated iodine with an excess of sodium sulfite before proceeding with the extraction and color development.

Initially the test color was viewed by holding the test tube containing the benzene-Rhodamine B against a white background, and comparing it with a blank treated similarly. Using this method it was difficult for an inexperienced operator to detect antimony in very low concentrations. Therefore the writer modified the procedure, pipetting the benzene extract directly into the depression of a white spot plate, and carefully adding to it the drop of aqueous reagent. The reagent drop

remained intact in the center of the depression, and the complex could be seen diffusing into the benzene layer, its color contrasting sharply with the white background. This provides a very striking and effective demonstration of the spot test method of analysis.

The spot test procedure, as finally developed, is as follows: one drop of the test solution is placed in a test tube, five drops of 1:3 sulfuric acid and one drop of 10% potassium iodide are added, followed by a few milligrams of solid urea. If free iodine appears to be liberated, solid sodium sulfite should be added until the color is discharged. The solution is then extracted by shaking vigorously with one milliliter of benzene. The benzene layer is removed with a pipet and placed in the depression of a white spot plate, in the center of which a drop of 0.2% aqueous Rhodamine B is then placed. Interpretation of the test, based on the appearance of the violet complex in the benzene, is made within ten seconds. The blank will have only a very faint pinkish hue.

A systematic study of interferences to this procedure was made, after the manner suggested by West (53). The ions studied were present in a concentration ratio of 1000 micrograms of the ion to 10 micrograms of antimony. The ions studied are shown in Table I. No interferences were found at this ratio. It was found that bismuth, at 1000 micrograms, gave a test color that might have been mistaken for antimony at 0.2 micrograms.

The determination of the limit of identification and concentration limit of the procedure was carried out in the manner prescribed by Feigl (18). They are as follows:

TABLE I

Scope of Interference Studies

I	II	III	IV	V	VI	VII	VIII	Miscellaneous
Li^{+}	Be^{++}	BO_2^{-}	CO_3^{--}	NH_4^{+}		F^{-}	Fe^{++}	CN^{-}
Na^{+}	Mg^{++}	$B_4O_7^{--}$	SiO_3^{--}	NO_2^{-}	$S_2O_3^{--}$	Cl^{-}	Fe^{+++}	$Fe(CN)_6^{---}$
K^{+}	Ca^{++}	Al^{+++}	Ti^{++++}	NO_3^{-}	SO_3^{--}	ClO_3^{-}	Co^{++}	$Fe(CN)_6^{----}$
Cu^{++}	Zn^{++}	Ga^{+++}	Zr^{++++}	$H_2PO_2^{-}$	SO_4^{--}	ClO_4^{-}	Ni^{++}	NCS^{-}
Rb^{+}	Sr^{++}	In^{+++}	Sn^{++}	HPO_3^{--}	Cr^{+++}	Mn^{++}	$RuCl_5^{--}$	
Ag^{+}	Cd^{++}	La^{+++}	Sn^{++++}	$P_4O_{13}^{------}$	$Cr_2O_7^{--}$	MnO_4^{-}	$RhCl_6^{---}$	
Cs^{+}	Ba^{++}	Tl^{+}	Ce^{+++}	$P_6O_{18}^{------}$	SeO_3^{--}	Br^{-}	$PdCl_4^{--}$	
$AuCl_4^{-}$	Hg^{++}		Pb^{++}	PO_3^{-}	SeO_4^{--}	BrO_3^{-}	OsO_5^{--}	
	Hg^{+}		Th^{++++}	HPO_4^{--}	MoO_4^{--}	I^{-}	$IrCl_6^{---}$	
				$P_2O_7^{----}$	TeO_3^{--}	IO_3^{-}	$PtCl_6^{--}$	
				VO_3^{-}	TeO_4^{--}	ReO_4^{-}		
				$HAsO_3^{--}$	WO_4^{--}			
				$HAsO_4^{--}$	UO_2^{++}			
				Bi^{+++}	UO_4^{--}			

Limit of Identification: 0.2 microgram of antimony

Concentration Limit: 1:300,000

The effects of changing the reaction conditions were studied, and it was found that neither the acid concentration, nor the iodide concentration, nor the reagent concentration are in any way critical.

The procedure was carried out in concentrations of sulfuric acid varying from 4 N (1:8) to 18 N (1:1) without adversely affecting the sensitivity. At higher acid concentrations the air-oxidation of the acidic iodide solution is greatly accelerated, however, and if it is desired to eliminate the addition of sulfite the acidity should be kept below 12 N. If the acidity falls much below 4 N the sensitivity of the procedure is diminished.

The concentration of the potassium iodide solution added was varied from 2% to 20%, in increments of 2%, without decreasing the sensitivity. Higher concentrations of the iodide are of course less stable, and are less convenient on that account.

Rhodamine B concentrations of 0.02%, 0.05%, 0.1%, and 0.5% were tested. No effect on the sensitivity of the procedure was observed.

III. The Spectrophotometric Determination of Antimony

The standard solutions of antimony in 1:3 sulfuric acid, described above, were used throughout this phase of the work, except as noted in the second paragraph below.

To one milliliter of a solution containing 10 micrograms of antimony was added 0.1 milliliter of 10% potassium iodide. This solution was successively extracted with three 1.5 milliliter portions of benzene. The benzene extracts were removed by pipet and combined, and to them was

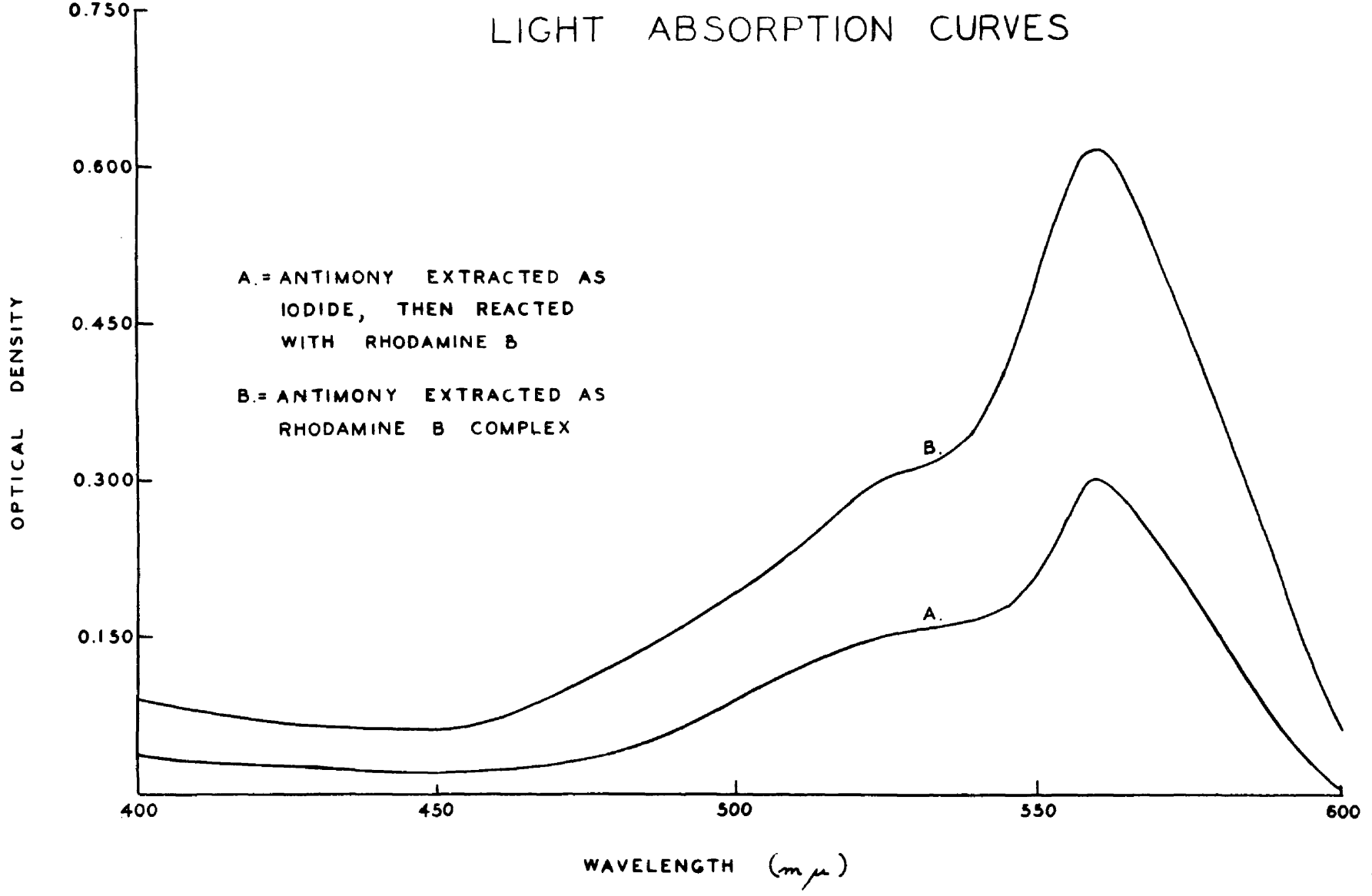
added 0.1 milliliter of a 0.2% aqueous solution of Rhodamine B. After thorough shaking the colored benzene layer was removed from the reagent; complete removal was insured by washing the reagent with two 1.5 milliliter portions of benzene. The combined colored benzene layer and washings were placed directly in a 10 milliliter volumetric flask, and the solution was diluted to the mark with benzene. The absorption spectrum of the benzene solution was then observed, using a Beckman Model DU spectrophotometer. The curve is shown in Figure 1. (A)

To one milliliter of a solution containing 20 micrograms of antimony, as the trichloride, in concentrated hydrochloric acid, was added one drop of 10% sodium nitrite solution, to oxidize the antimony to the pentavalent state. This solution was warmed for five minutes to destroy the nitrite, was cooled, and 0.1 milliliter of 0.2% aqueous Rhodamine B solution was added. The antimony-Rhodamine B complex was then extracted from the solution by shaking with three successive two milliliter portions of benzene. The extracts were combined and placed in a ten milliliter volumetric flask, then diluted to the mark with benzene. The absorption spectrum of this solution was then observed, using a Beckman Model DU spectrophotometer. The resultant curve is shown in Figure 1. (B)

To test the conformity of the colored complex to Beer's Law, the procedure was applied to four solutions of widely varying antimony concentration. One milliliter volumes of solutions containing 5, 10, 20, and 40 micrograms of antimony per milliliter were delivered from a calibrated pipet into test tubes. To each was added 0.1 milliliter of 10% potassium iodide. The solutions were then shaken vigorously with

FIGURE 1

LIGHT ABSORPTION CURVES



three successive 1.5 milliliter portions of benzene, the benzene layers being removed by pipet and combined in separate test tubes. To each of the tubes containing the combined extracts was added 0.1 milliliter of 0.2% aqueous Rhodamine B. The tubes were thoroughly shaken, the colored benzene layers removed, the reagent washed with two 1.5 milliliter portions of benzene, and the combined washings and colored benzene layers were put into 10 milliliter volumetric flasks and diluted to the mark with benzene. Absorption spectra were then observed for these four solutions, using a Beckman Model B spectrophotometer. The resultant curves are shown in Figure 2.

Inasmuch as the rather sharp absorption maximum at 560 millimicrons did not shift or diminish in intensity with varying antimony concentration, the relationship of optical density to concentration was investigated over a range of concentrations. The procedure employed was the same as that described immediately above. The resultant curves are shown in Figures 3-4. Data for these curves are given in Tables II-III.

The effect of variation of conditions upon the procedure was next investigated. The procedures employed in the extraction and color development were the same as those described above.

The stability of the color of the complex, at a concentration of 10 micrograms of antimony per milliliter, was observed over a period of 3.5 hours. The color was found to be altogether stable during this period. The same solution examined ten hours later, was found to have decreased in intensity only slightly. The results are shown in Figure 5.

The effect of varying the acidity of the sample was studied by preparing solutions containing 10 micrograms of antimony in sulfuric acid

FIGURE 2

ABSORPTION CURVES FOR SEVERAL CONCENTRATIONS OF ANTIMONY

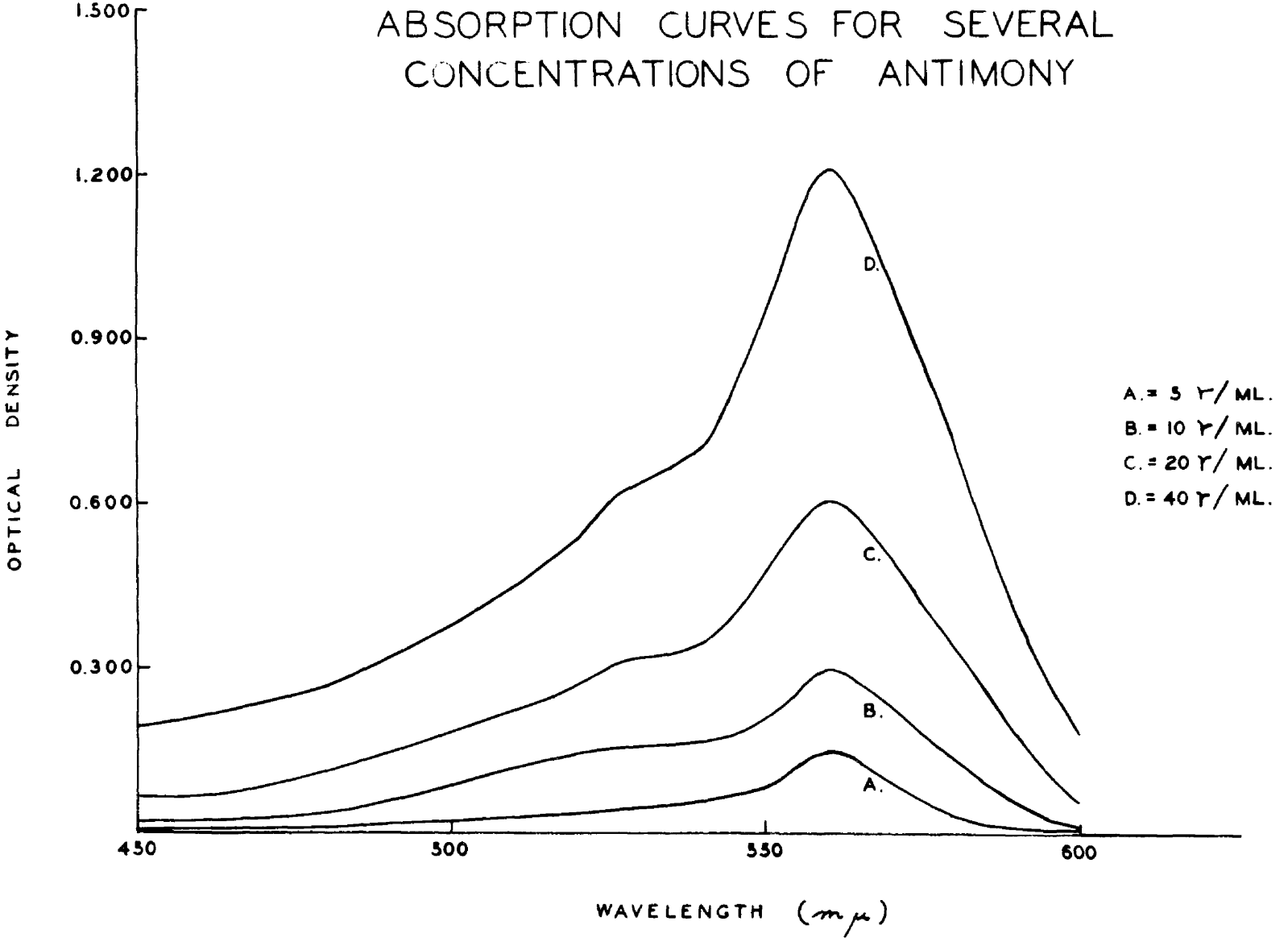


TABLE II

Data for Concentration Curve (Figure 3)

Antimony Concentration (micrograms/milliliter)	Optical Density (at 560 mμ)
5	0.160
10	0.300
15	0.452
20	0.568
25	0.761
30	0.879

FIGURE 3

CONCENTRATION CURVE

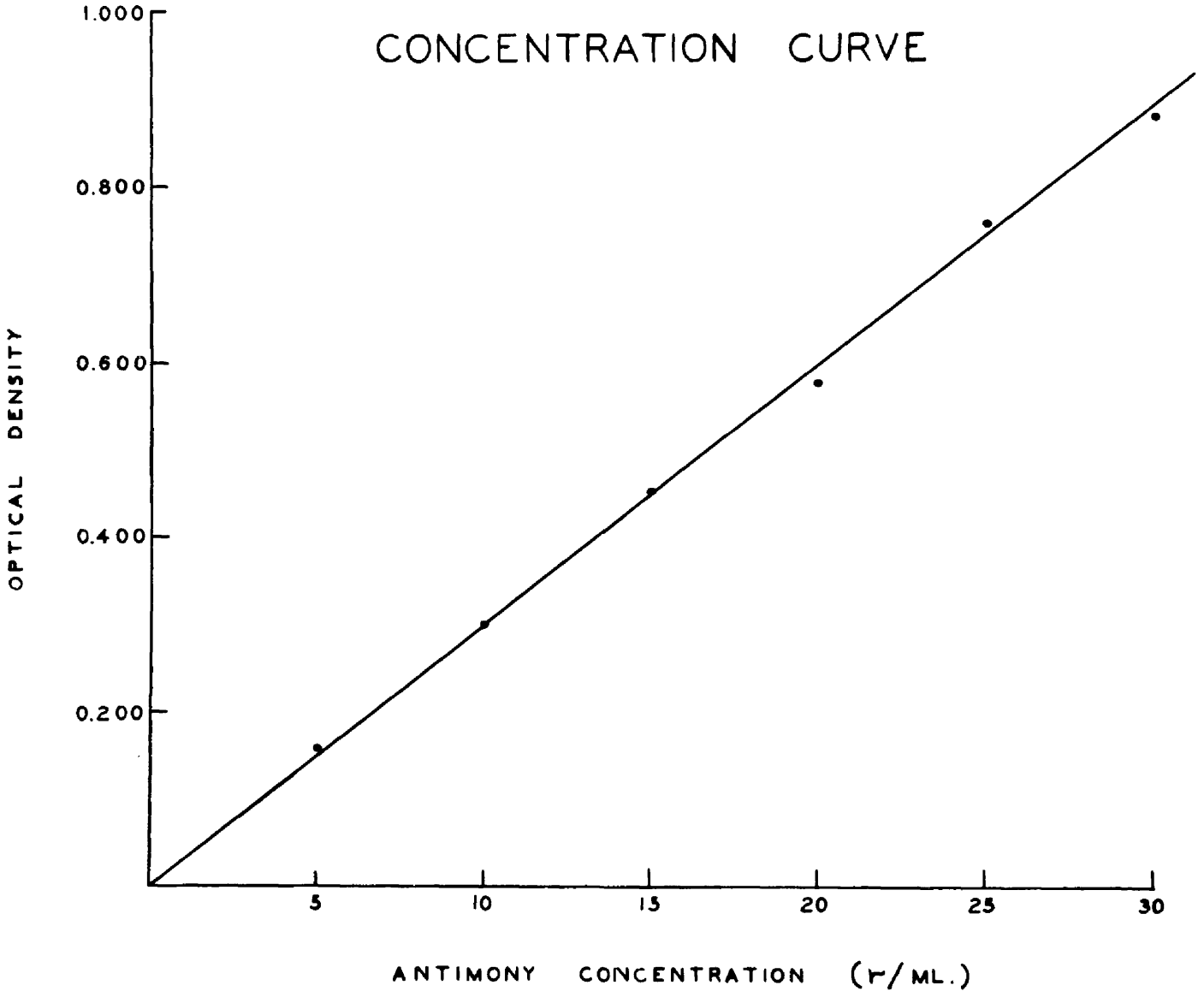


TABLE III

Data for Concentration Curve (Figure 4)

Antimony Concentration (micrograms/milliliter)	Optical Density (at 560 mu)
1	0.040
2	0.060
3	0.103
4	0.131
5	0.151
6	0.180
7	0.204
8	0.255
9	0.275
10	0.295

FIGURE 4

CONCENTRATION CURVE

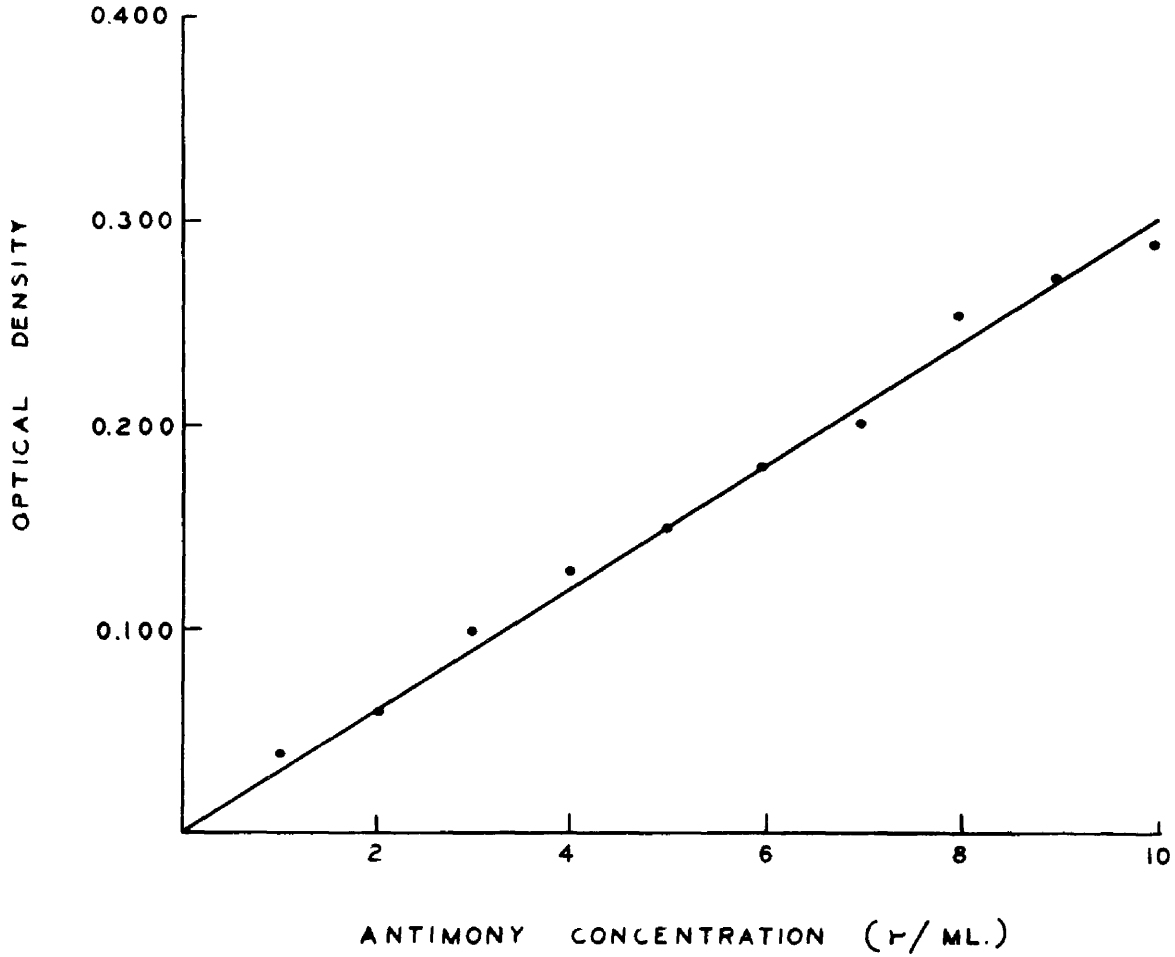
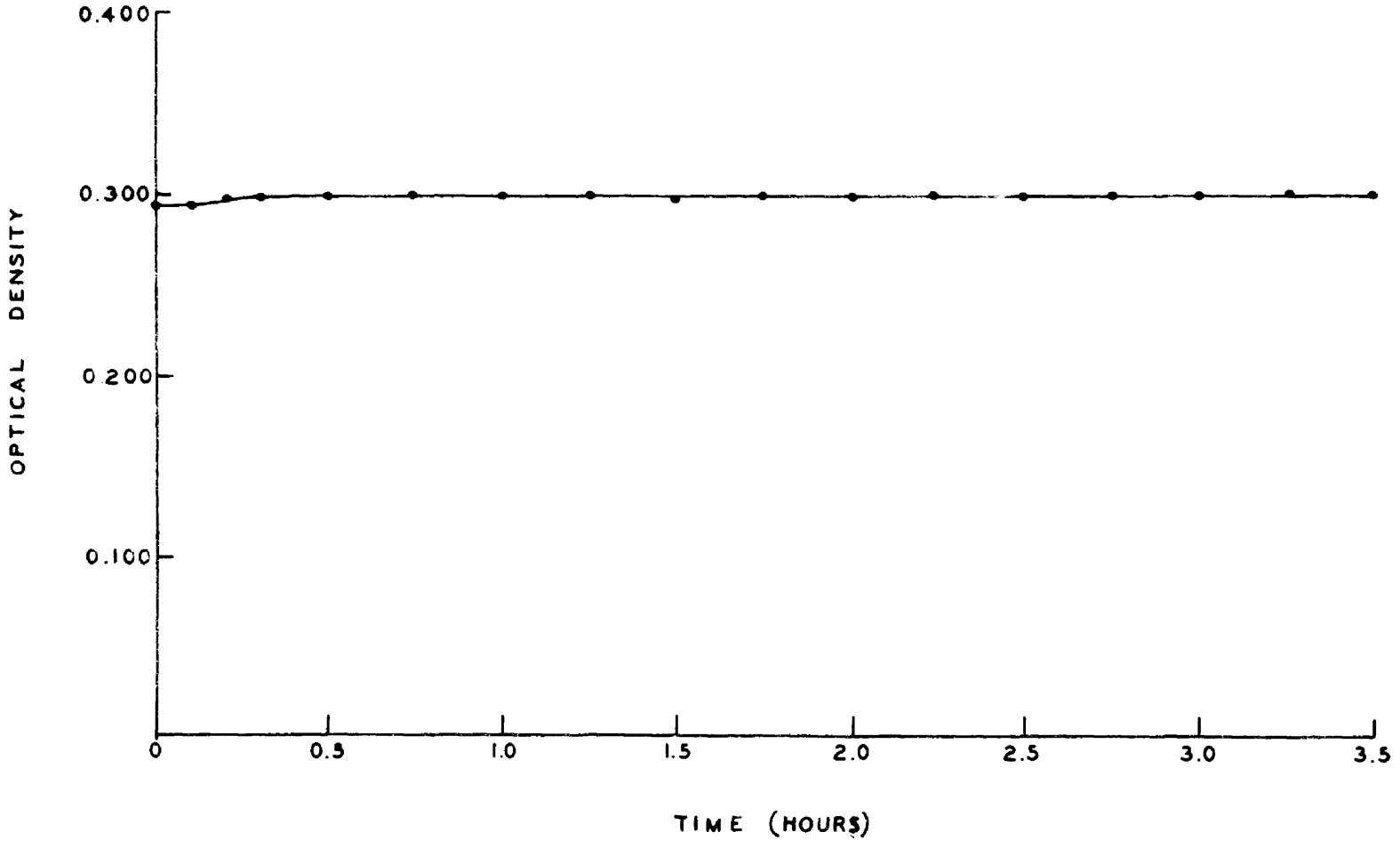


FIGURE 5

COLOR STABILITY OF
ANTIMONY - RHODAMINE B COMPLEX



whose strength was varied from 1 N to 18 N. It was found that a considerable range of acidity was permissible. Variations from 4 N to 12 N in sulfuric acid did not cause appreciable changes in optical density. At acidities much greater than 12 N an increase in optical density was noted, possibly due to the accelerated air-oxidation of the iodide solution. For acidities of less than 4 N the optical density began to decrease, probably because of lessened extraction. Figure 6 shows graphically the effect of acidity upon final optical density.

The ratio of iodide ion to antimony was altered systematically to study its effect upon the optical density, and to possibly gain some evidence of the nature of the antimony-iodide complex being extracted. As shown in Figure 7, a rapid change in optical density occurs as the iodide/antimony molar ratio is increased, until the value 10/1 is reached. This ratio is of no obvious theoretical significance.

The effect of the Rhodamine B concentration was also investigated, by varying the ratio of mols Rhodamine B to mols antimony in the initial solution. The results, shown in Figure 8, are again of no obvious significance.

Inasmuch as the ordinary fluctuations in laboratory temperatures were not observed to affect the reproducibility of the method, no study of temperature effects was made.

A detailed study of interferences to the procedure was made. The list of ions studied is given in Table 1. These ions were present in the amount of 1000 micrograms, as compared to 10 micrograms of antimony. If the relative error did not exceed 5%, the ion was considered as not constituting an interference. Nitrate and nitrite were

FIGURE 6

EFFECT OF ACID CONCENTRATION UPON OPTICAL DENSITY

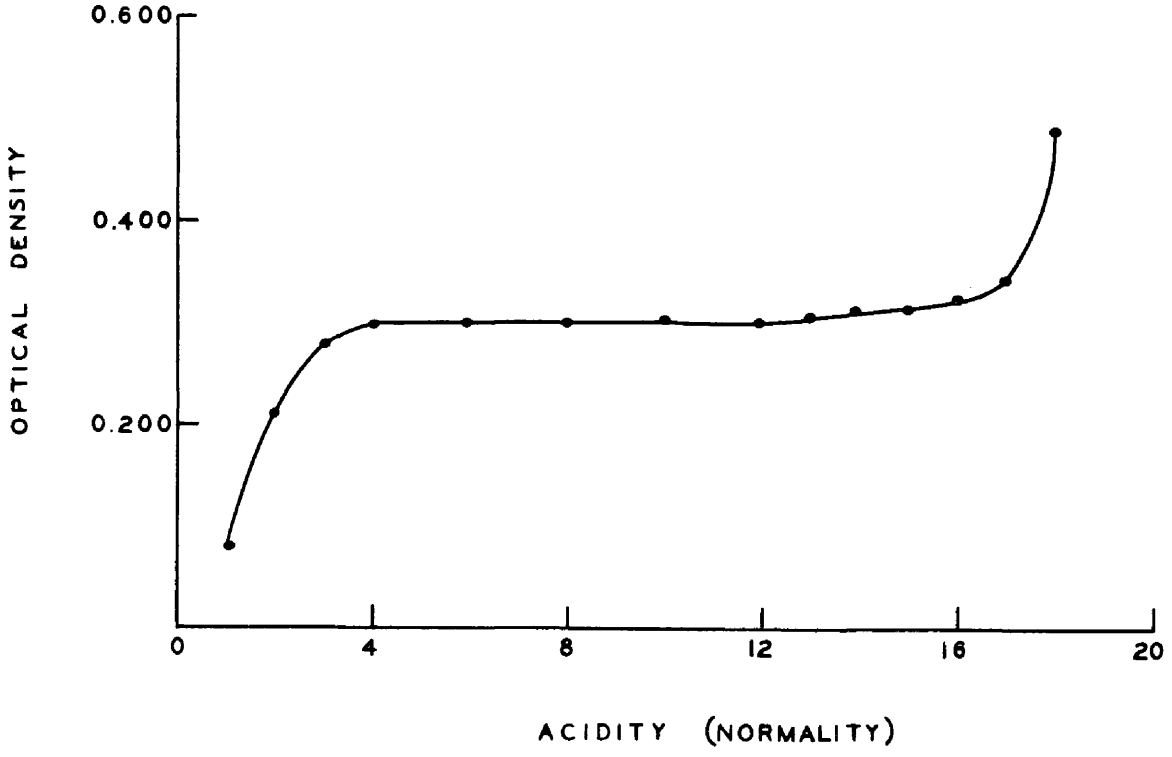


FIGURE 7

EFFECT OF IODIDE CONCENTRATION
UPON OPTICAL DENSITY

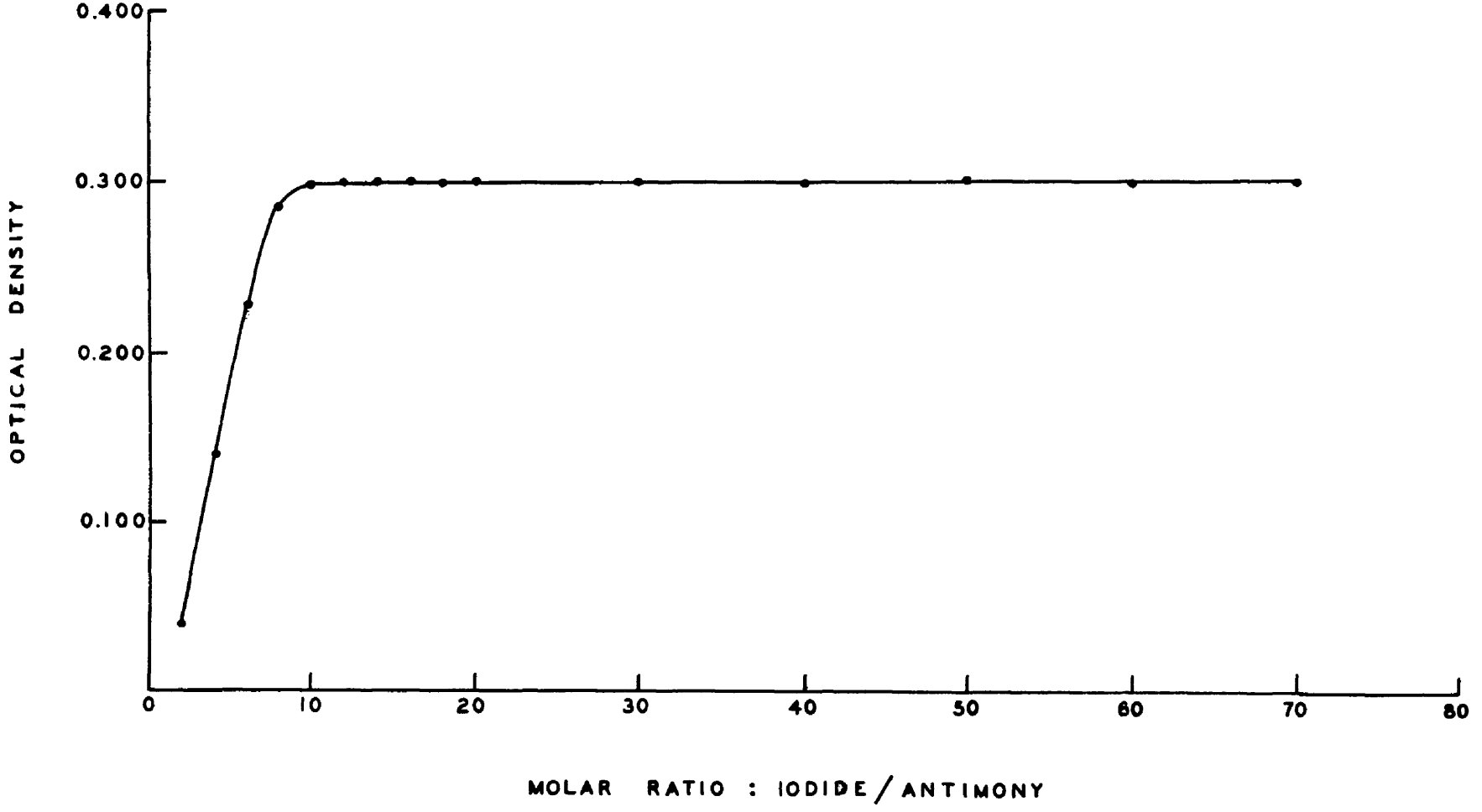
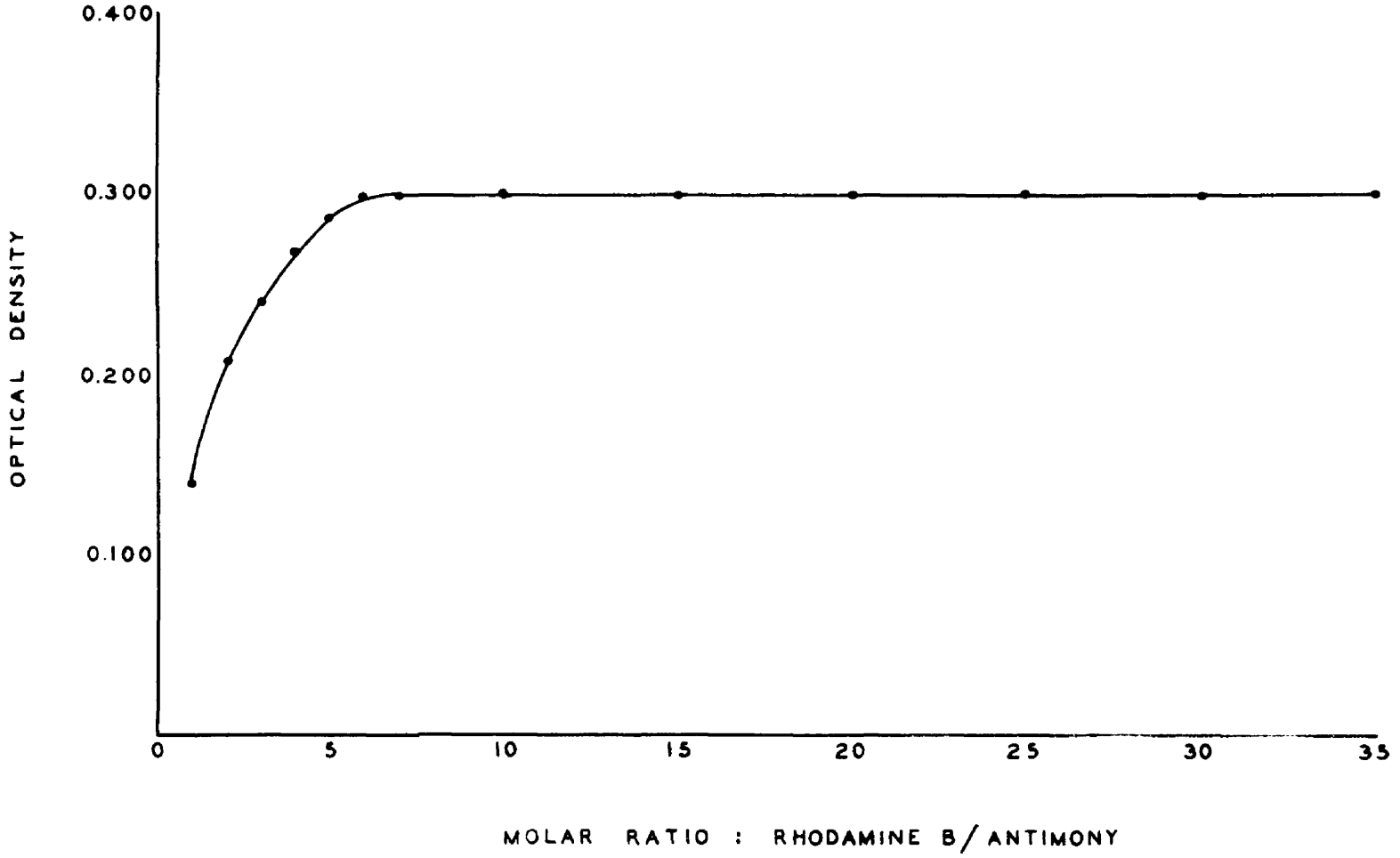


FIGURE 8

EFFECT OF RHODAMINE B CONCENTRATION UPON OPTICAL DENSITY



removed prior to the procedure by evaporating the solution to fumes of sulfur trioxide, cooling, and re-diluting to the initial volume. The use of urea to remove these interferences lessened the reproducibility of the method. The addition of solid sodium sulfite was successfully used to eliminate the interference due to the liberation of free iodine by such oxidizing agents as permanganate, bromate, ferric and cupric ions. The use of sulfite lessened, although not seriously, the reproducibility of the method. Bismuth was the only ion whose interference it was not possible to eliminate by the above two steps. In the ratio given above, it caused a relative error of 21%. Bismuth can readily be removed from antimony by extraction with dithizone if desired.

Over the range 0 - 200 micrograms of antimony, three successive extractions with benzene were required to remove completely the antimony from the aqueous layer.

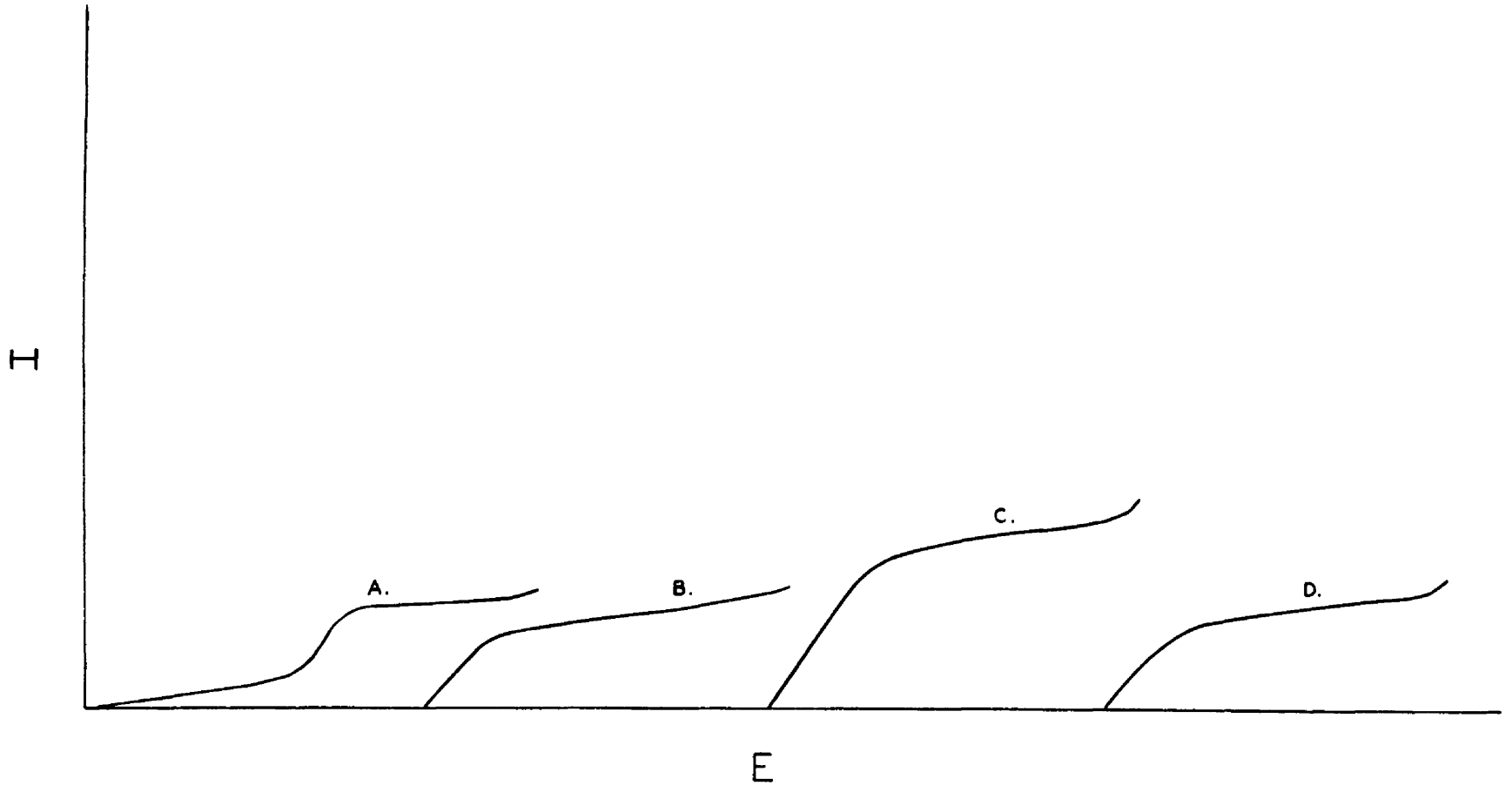
A spectrographic survey of the extraction was made. In this study 1000 micrograms of each of the spectrum-emitting ions covered in the interference study were taken, in groups, according to Table 1, and the extraction procedure was carried out in the usual fashion. Graphite electrodes were then immersed in the benzene extracts for twenty-four hours. The spectra emitted by these electrodes, using an arc source, were then photographed, along with the iron spectrum, a raies ultimes spectrum, and a blank. Bismuth was the only ion observed to be extracted, and its lines were extremely faint.

The fact that Rhodamine B had heretofore been reported as reacting with only antimony (V), and not antimony (III), plus the fact that chloride ion had always been reported as absolutely essential to the reaction, made imperative an investigation of the mechanism of

the extraction. It was difficult to see how antimony (III) would be oxidized under the prevailing conditions; the trivalent antimony standards used had been reported by Maren (35) and other workers as being stable for two years or more. Accordingly a polarographic investigation of the valence state of antimony in the benzene layer was carried out. Kolthoff and Lingane (33) describe a well-defined wave existing for trivalent antimony in 1 N sulfuric acid, with methylene blue as a maximum suppressor, but state that pentavalent antimony gives no wave under these conditions. 100 micrograms of the antimony (III) standard in 1:3 sulfuric acid was diluted to 9.0 milliliters with water, giving an acid concentration of 1 N. Methylene blue was in sufficient amount to make its concentration 0.01%. The system was purged of oxygen in a polarographic cell with nitrogen, and then polarographed at a shunt setting of 50, using a dropping mercury electrode. The curve obtained is shown by "A" in Figure 9. Next, to 100 micrograms of antimony (III) in 9.0 milliliters of 1 N sulfuric acid there was added 0.1 milliliter of 10% potassium iodide, followed by the above amount of methylene blue. This solution was polarographed under identical conditions with the first. The results are shown in curve "B" of Figure 9. Following this, 100 micrograms of antimony (III) in 1:3 sulfuric acid were extracted in the usual fashion, the extract was placed in a beaker over 9.0 milliliters of 1 N sulfuric acid and the benzene was flashed off. The solution was cooled, methylene blue was added, and the solution polarographed under the same conditions as the previous two had been. Curve "C" in Figure 9 shows the result. The procedure just given was then repeated, except that 0.1 milliliter of 10% potassium iodide was also added to the sulfuric acid solution after the benzene had been flashed off. Curve "D" in Figure 9 is

FIGURE 9

POLAROGRAMS OF VARIOUS ANTIMONY SOLUTIONS



the resultant curve.

DISCUSSION OF RESULTS AND CONCLUSIONS

The reactions encountered in the spot test procedure were most surprising. It was altogether contrary to expectation that benzene should have the power to extract the iodide complex from aqueous solution. As already mentioned, the reaction between Rhodamine B and antimony has always been described as requiring the pentavalent form of the ion, whereas in these studies it seemed certain that the trivalent form was reacting. And finally, chloride ion, which had been reported by every prior investigator as absolutely essential to the reaction between antimony and Rhodamine B, was not present in the procedure at hand.

The sensitivity and selectivity of the spot test procedure described here leave little to be desired. Segriwe (11) had reported 0.5 micrograms as the limit of identification of the Rhodamine B procedure when used in an aqueous system. The increased sensitivity observed by the writer is not surprising, as metallo-organic compounds are frequently more highly colored in organic solvents than in water.

The use of the extraction procedure, plus the use of urea and sodium sulfite prior to the extraction, permit the elimination of all interferences to the test. This constitutes a striking illustration of how the hope for specificity in spot test analysis lies, not in the reagent, but in the procedure. Rhodamine B, although a sensitive and otherwise desirable reagent, is far from being a specific reagent for antimony. Yet, by the proper control of the test conditions, and by

applying the technique of extraction, specificity has been attained.

The great similarity of the absorption curves obtained on the one hand by extracting trivalent antimony as the iodide into benzene, and reacting it there with Rhodamine B, and on the other by reacting pentavalent antimony in aqueous solution with Rhodamine B and chloride ion, indicates that the colored compounds formed are closely similar in nature. No evidence for the structure of this compound had ever been found by prior investigators, although attempts had been made to do so. The writer's polarographic investigation seems to show strong evidence that the antimony in the benzene extract is in the trivalent state. Curve "A" in Figure 9 is the curve described by Kolthoff and Lingane (33) for antimony (III) in 1 N sulfuric acid. Curve "B" indicates the effect of having antimony (III) present as the iodide complex; the decomposition potential of the antimony is so shifted that the reduction begins immediately upon the application of potential to the system. Iodide ion, it should be noted, is present here in considerable excess. In curve "C", where the extraction has been performed, it is noted that again the decomposition begins immediately but that the step height of the curve is considerably greater. In this case iodide is not present in excess, having presumably been extracted only in the storchiometric ratio required by the antimony tetraiodide complex. In curve "D" an excess of iodide was added to the solution after the extracted benzene layer had been flashed off. This excess of iodide should be quite close to that existing in curve "B", and the similarity of the magnitude of the step heights in "D" and "B" indicates that this is dependent upon the amount of excess iodide present.

While benzene has been very frequently used to extract metallo-organic complexes, this appears to be the first time it has been used in an analytical procedure to extract an inorganic compound or complex. The fact that the extraction falls off sharply when the iodide/antimony molar ratio becomes less than 10:1 is without obvious significance; certainly antimony does not possess a coordination number of ten. Nor does the Rhodamine B/antimony molar ratio for maximum optical density give any clue as to the nature of the colored compound formed. The effect of the acidity upon the efficiency of extraction is not surprising, this being the rule rather than the exception.

As to the procedure itself, there is much to recommend it. The fact that the antimony is present in the trivalent state is a considerable advantage. Maren (35) has shown rather conclusively that in oxidizing antimony (III) to antimony (V) a certain amount of antimony (IV) will probably be formed. This "chemical entity" as Maren calls it, is nearly impossible to oxidize, although it is easily reduced with sulfite. Maren was able to circumvent this difficulty only by using perchloric acid as an oxidant, a step which many analysts prefer to avoid. A certain tedium is involved in extracting three times, but this is more than compensated for by the immense gain in specificity. The color stability, the adherence to Beer's Law, the wide permissible concentration ranges of acid, iodide, and Rhodamine B, the low cost and stability of the reagents, and the accuracy and precision of the method all recommend its widespread adoption.

SUMMARY

1. A specific spot test procedure for the detection of antimony has been developed, based on the extraction of antimony (III) as the iodide complex into benzene, and consequent reaction of the extract with a solution of Rhodamine B. The reaction product is formed in the benzene layer, to which it imparts a characteristic color. This procedure will detect with certainty as little as 0.2 microgram of antimony, at a concentration of 1:300,000. Specificity is gained by the addition of urea and sodium sulfite, and by virtue of the extraction procedure. Based on the criteria of sensitivity, selectivity, ease of performance, non-critical nature of test conditions, and cost, stability and availability of reagents, it appears superior to any test yet reported for the detection of antimony.

2. On the basis of the above reaction, a spectrophotometric procedure has been devised for the estimation of microgram quantities of antimony. Antimony (III), in 1:3 sulfuric acid is reacted with potassium iodide. The resultant iodide complex is quantitatively removed from the aqueous phase by extraction with three successive portions of benzene. The benzene extract is reacted with an aqueous solution of Rhodamine B, the colored reaction product being formed in the benzene layer, which is removed, diluted to volume, and measured at 560 millimicrons. Interfering ions, if present, can be successfully eliminated by treatment prior to extraction. Bismuth is the sole exception to this, and its interference even at a ratio to antimony of 100:1 is

not serious. At 10 micrograms of antimony the relative analysis error of the procedure is 5.0%.

3. The stability of the colored compound has been studied. The conditions of acidity, iodide concentration, and Rhodamine B concentration necessary for the success of the procedure have been defined.

4. A spectrographic survey of the extraction indicates that bismuth is the only other metal extracted under the conditions given.

5. Polarographic studies give evidence that the antimony is present in the benzene extract in the trivalent state.

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VITA

William C. Hamilton was born in Marietta, Pa. on October 19, 1920. He received his elementary education in the grade school of that town, and graduated from Marietta High School in 1938, as valedictorian of his class. In the fall of the same year he entered Franklin and Marshall College and was graduated in the spring of 1942 from that institution with a B. S. degree in chemistry.

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On October 28, 1944 he was married to Claudia Marie Lafleur of Ville Platte, La. They have two sons.

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